Improving quantitative laboratory analysis of phycobiliproteins to provide high quality validation data for ocean color remote sensing algorithms

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Abstract

Identification and characterization of phytoplankton communities and their physiology is a primary aim of NASA's PACE satellite mission. The concentration and composition of phytoplankton pigments modulate the spectral distribution of light emanating from the ocean, which is measured by ocean color satellites, and thus provide critical information on phytoplankton community composition and physiological parameters. One diagnostic class of pigments not routinely well-characterized is the phycobiliproteins (PBPs), and NASA has a requirement to collect and distribute high quality *in situ* data in support of data product validation activities for ocean color missions. Phycobiliproteins are light-harvesting proteins that are the predominant photosynthetic pigments in some classes of phytoplankton including cyanobacteria, such as *Synechococcus*, *Trichodesmium*, and *Microcystis*. With the advance of hyperspectral ocean color sensors such as on PACE (expected to launch in late 2022), it is essential that we implement routine analysis of PBPs that satisfies several considerations: reproducible, high extraction efficiency for a variety of environments, and Suitable for large scale analysis. Published techniques for PBP analysis vary in recommendations for: collection, extraction, disruption mode, and analysis; evidence suggests the variation in results may depend at least in part on the species and even strain(s) of interest. Experiments that tested variations in these parameters have drawn very different conclusions regarding extraction efficiency and reproducibility. Cyanobacteria are more difficult to extract than other PBP-containing algae such as cryptophytes, but can be important primary producers. We used a cryptophyte (*Rhodomonas salina*) and cyanobacterium (*Synechococcus* sp.) to compare extraction efficiencies of water samples concentrated via centrifugation to filtered samples using two different extraction buffers (phosphate and asolectin-CHAPS). Samples were analyzed on a fluorometer configured for phycoerythrin (PE) detection. The results have important implications for collection and storage of samples for routine analysis; some previous studies (although not all) have suggested that filtered samples have a much lower extraction efficiency than whole water samples.

Background

In order to support next generation ocean color missions such as PACE, we need to quantitatively understand phycobiliprotein (PBP) contributions to the satellite signal. Phycocyanins (PC, absorbance range 610-620 nm) are typically associated with freshwater environments and phycoerythrins (PE, absorbance range 540-570 nm) are typically associated with marine environments, although there can be significant overlap.

Goals:

- Implement routine analysis of PBPs that is: reproducible, has a high extraction efficiency, and is suitable to being implemented on a large scale.
- Test if one extraction methodology could be applied to samples from all environments

The Cultures:

Rhodomonas salina

(CCMP 1319, also known as 3C, NEPCC76, LB2423, and CS-174)

—Marine cryptophyte usually found in temperate regions

Synechococcus sp.

(CCMP 3074, also known as CC9902)

—Marine cyanobacteria usually found in coastal areas

—Part of subcluster 5.1A, which only contains PE

There is significant variation in suggested methodology for collection, extraction, and analysis of PBPs in the literature. Even among papers comparing techniques, different conclusions have been drawn regarding the best practices. In some instances, this is because the researchers have different goals (Ex. only interested in 1 pigment); others are not as easily

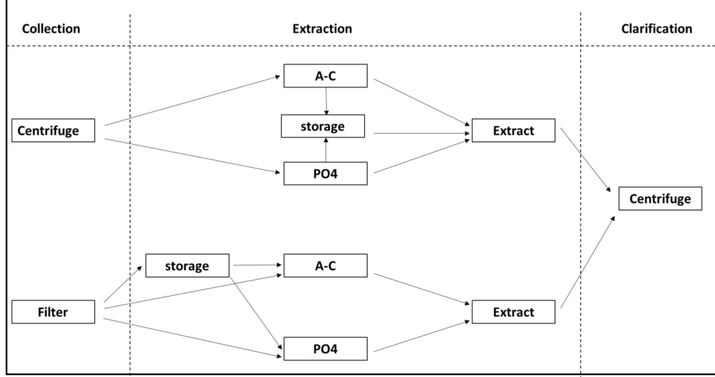
Summary of previous research comparing techniques for quantitative PBP extraction

Author	Collection method		Buffer			Disruption method			
	Centrifuge	Filter	PO4	A-C	Other	Freeze/Thaw	Sonicate	Grind	Other
Stewart & Farmer 1984		X			lysozyme			X	
Wyman 1992	X	X	X				X	X	French press
Viskari and Colyer 2003	X	X	X	X	lysozyme, 5 unique buffers	X	X	X	French press, N2 bomb
Silveira et al. 2007		X	X		4 unique buffers				elevated heat
Zhu et al. 2007	X		X		lysozyme,	X	X	X	
Lawrenz et al. 2010	X	X	X			X	X	X	
Zimba 2012		X	X	X X		X	X		
Horvath et al. 2013	X	X	X			X	X	X	
Sobiechowska-Sasim et al. 2014		X			lysozyme			X	
Yacobi et al. 2015		X	X		lysozyme			X	
Thoisan et al. 2017		X	X			X	X		lyophilization

Methodology

We tested several methodological variables:

- **collection method**-centrifugation, filtration
- **buffer type**-100mM phosphate (PO_4) and modified asolectin -CHAPS (A-C)
- **storage**-extracted immediately or stored in liquid nitrogen



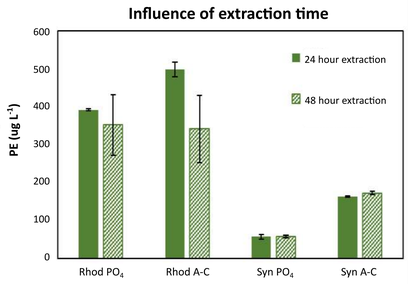
Notes:

- There were three replicates of each treatment.
- 5 ml buffer was added to each sample. After ultra-sonication with a microtip, 1 ml buffer was used to rinse the tip into the sample.
- Samples collected by centrifugation were frozen (-20°C) after buffer was added, thawed, sonicated, frozen again (-20°C), then thawed overnight at -4°C.
- Filtered samples were sonicated, frozen (-20°C), then thawed overnight at -4°C.
- All filter samples were collected on GF/Fs and flash frozen, whether extracted immediately or stored in liquid nitrogen for later analysis.
- We opted to use modified A-C buffer (Zimba 2012) as opposed to the original formulation (Viskari and Colyer 2003), based on preliminary testing.
- Samples were analyzed on a Turner Designs 10-AU fluorometer calibrated and configured for PE analysis.
- Statistical comparisons were made using 2-tailed t-tests.

Results

Testing extraction time:

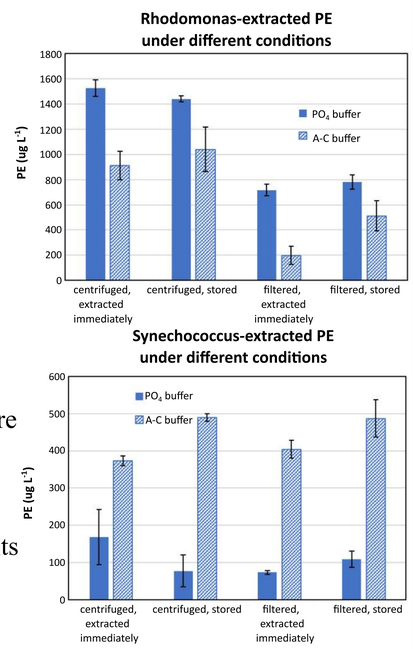
Filtered samples were extracted for either 24 or 48 hours. The 48 hour samples underwent an additional extraction cycle (freeze/thaw and sonication). There was no significant difference ($p>0.05$) between results for samples extracted for 24 hours compared to those extracted for 48 hours, although there is some indication *Rhodomonas* extraction may start to degrade after 24 hours (increased standard deviation, decreased mean concentration). In preliminary testing, we saw similar results with *Trichodesmium* and a different batch of *Rhodomonas* (data not shown).



Impact of collection method, storage, and buffer choice:

- There is no significant difference ($p>0.05$) between storing samples or analyzing them immediately, when comparing samples from the same collection technique. Therefore the preservation methods employed (freezing in liquid nitrogen or immediately centrifuging and adding buffer before freezing at -20°C) were effective, as PBPs can start to degrade almost immediately upon the death of the cell (Stewart and Farmer 1984).
- There is a significant difference ($p<0.05$) between the extraction capabilities of PO_4 and A-C buffers, except for frozen *Rhodomonas* samples (visually, there appears to be a difference; the lack of significance may result from the small sample size and high variance). PO_4 buffer had higher extraction efficiency than A-C buffer for *Rhodomonas*; the opposite was true for *Synechococcus*. These results are consistent with what was seen in the extraction time experiment.
- There is a significant difference between samples collected via centrifugation and samples collected on filters, with filtered samples exhibiting lower extraction efficiency regardless of culture. These results are consistent with results seen by Lawrenz et al. (2011).

(Note: when calculating statistics, results from centrifuged *Synechococcus* samples extract in PO_4 and analyzed immediately were not included as one sample cracked during extraction).



Conclusions/Future directions

Based on these tests, cryptophytes and cyanobacteria need different extraction buffers. Centrifuged samples offered better extraction efficiency compared to filtered samples, but this type of sample collection is not practical on a large scale. Further research is needed to develop a filtration-based method that offers equivalent extraction.

We intend to produce standardized protocols for PBP analysis. In order to do that, we must quantify the uncertainties of phycobiliprotein analysis and develop methodologies that can be practically implemented on a large scale.

Future work:

- Grow cultures in larger batches for more replicate samples (statistics limited by small sample size).
- Test other extraction media, and how long samples remain viable in frozen storage.
- Test other types of filters for sample collection to improve extraction efficiency.
- Test procedures with other cultures (*Trichodesmium*, *Prochlorococcus*, other *Synechococcus* strains) and natural samples expected to contain PBPs.

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References

Horváth H, et.al. Extraction methods for phycocyanin determination in freshwater filamentous cyanobacteria and their application in a shallow lake. *Eur J Phycol*. 2013; 48(3):278-286. doi:10.1080/09670262.2013.821525
Lawrenz E, Fedewa EJ, Richardson TL. Extraction protocols for the quantification of phycobilins in aqueous phytoplankton extracts. *J Appl Phycol*. 2011; 23(5):865-871. doi:10.1007/s10811-010-9600-0
Silveira, S.T. et.al., Optimization of phycocyanin extraction from *Spirulina platensis* using factorial design. *Bioresource Technology*. 2007; 98(8):1629-1634. doi: 10.1016/j.biortech.2006.05.050
Sobiechowska-Sasim M, et al. Quantitative analysis of extracted phycobilin pigments in cyanobacteria—an assessment of spectrophotometric and spectrofluorometric methods. *J Appl Phycol*. 2014; 26(5):2065-2074.
Stewart DE, Farmer FH. Extraction, identification, and quantitation of phycobiliprotein pigments from phototrophic plankton. *Limnol Oceanogr*. 1984; 29(2):392-397. doi:10.4319/lo.1984.29.2.0392
Thoisen, C., Hansen, B. W. and Nielsen, S. L. A simple and fast method for extraction and quantification of cryptophyte phycoerythrin. *Methods X*. 2017; 4209–213. doi: 10.1016/J.MEX.2017.06.002
Viskari PJ, Colyer CL. Rapid extraction of phycobiliproteins from cultured cyanobacteria samples. *Anal Biochem*. 2003; 319(2):263-271. doi:10.1016/S0003-2697(03)00294-X
Wyman M. An *in vivo* method for the estimation of phycoerythrin concentration in marine cyanobacteria (*Synechococcus* spp.). *Limnol Oceanogr.* 1992; 37:1300–1306. doi: 10.4319/lo.1992.37.6.1300
Yacobi YZ, Köhler J, Leunert F, Gitelson A. Phycocyanin-specific absorption coefficient: Eliminating the effect of chlorophylls absorption. *Limnol Oceanogr Methods*. 2015; 13(4):157-168. doi:10.1002/lom3.10015
Zhu Y, Chen XB, Wang KB, et al. A simple method for extracting C-phycocyanin from *Spirulina platensis* using *Klebsiella pneumoniae*. *Appl Microbiol Biotechnol*. 2007; 74(1):244-248. doi:10.1007/s00253-006-0636-7
Zimba P V. An improved phycobilin extraction method. *Harmful Algae*. 2012; 17(March):35-39. doi:10.1016/j.hal.2012.02.009